The Role of Amygdala Norepinephrine in Memory Formation: Involvement in the Memory Enhancing Effect of Peripheral Epinephrine

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Abstract

The present study examined the roles of amygdala α_1 and β noradrenergic receptors in memory formation as well as their involvement in the memory enhancing effect of peripheral epinephrine (E). Male Sprague-Dawley rats with cannulae implanted into the amygdala were trained on the one-trial inhibitory avoidance task and tested for retention 24 hrs later. Immediately after training, they received various treatments to alter amygdala noradrenergic functions and/or peripheral adrenergic functions. Separate groups of animals were decapitated 10 min after training for assays of monoamine levels in various brain regions by the HPLC-EC method. Results indicated that, when infused into the amygdala immediately after training, isoproterenol and 8-bromo-cAMP enhanced retention, while propranolol impaired retention. On the other hand, phenylephrine or prazosin failed to produce statistically significant effects. Posttraining intra-amygdala infusion of propranolol, but not prazosin, abolished the memory enhancing effects of norepinephrine (NE) infused into the amygdala or E given subcutaneously to the adrenal demedullated rats. Depletion of amygdala NE by the selective neurotoxin DSP-4 also abolished the memory modulatory effects of E. These findings support that amygdala noradrenergic β, but not α_1, receptors are involved in both central and peripheral memory modulatory processes. However, since the postmortem tissue NE levels in the amygdala and other brain regions did not differ among various groups, the inhibitory avoidance training and peripheral E may only activate a transient functional increase in the amygdala NE activity. (Chinese J.Physiol. 38: 81-91, 1995)

Key Words: amygdala, norepinephrine, epinephrine, DSP-4, avoidance learning, memory.

Introduction

Central norepinephrine (NE) may subserve a role in memory formation because events leading to its release generally create strong and enduring memories (27). While certain treatments disrupting the global noradrenergic function yield conflicting effects on learning and memory (10, 42, 44, 46, 47, 52, 56), manipulation of NE functions in specific brain regions shortly after training is able to affect subsequent memory: In the inhibitory avoidance task, posttraining intra-amygdala infusion of β blockers—dl-propranolol or dl-alprenolol impairs retention (15), while posttraining intra-amygdala infusion of NE would, depending on the dose, enhance or impair retention (12). Infusion of NE into other brain regions has been shown to affect learning and memory in classical conditioning (55) and olfactory discrimination (20). The effects of NE or its blockers on memory are time-dependent (30): Treatments affect retention only when given shortly after training but have no effect if given several hours later. These findings suggest that NE may be naturally released in the amygdala during learning to facilitate memory consolidation processes.

The amygdala NE activity is also involved in the memory modulatory effects of other treatments (7, 24, 28) including that of epinephrine (E). Peripheral E is proposed to work as an endogenous memory modulator (17, 36): Avoidance learning releases E

into the plasma (38). Removal of the adrenal medulla either before or shortly after training produces a retention deficit (4, 6, 31, 45). At moderate doses (0.01 to 0.1 mg/kg), E given subcutaneously enhances memory in untreated rats (9, 17, 21) or attenuates the retention deficit in adrenal demedullated (ADMX) or adrenal denervated (ADNX) rats (4, 29), while at high doses (0.5 to 1.0 mg/kg) E impairs memory (33).

Circulating E in the periphery hardly crosses the blood-brain-barrier (58). Several lines of evidence suggest that its influence on memory may be mediated by amygdala NE. First, the presence or absence of peripheral E would alter the effect of posttraining amygdala electrical stimulation on memory (29, 37). Second, chemical lesions of the amygdala readily abolish memory enhancing effect of E (5). Third, posttraining intra-amygdala infusion of NE ameliorates the retention deficits in adrenal demedullated (ADMX) rats (30). Fourth, lesions of a major amygdala afferent-efferent pathway—the stria terminalis (ST)—block the memory enhancing effects of E injected systemically (32) or NE infused into the amygdala, while knife-cut transection of the ventral amygdalofugal pathway (VAF) attenuates the E effect but leave the NE effect intact (33). These findings are consistent with the possibility that peripheral E may somehow activate the brainstem NE projections to various brain regions, particularly those to the amygdala through the VAF; NE thus released may modulate memory processing through the output influences of the ST (39).

Both E and NE can activate α and β noradrenergic receptors. There is evidence that E enhances memory by stimulating both α and β receptors in the periphery (24, 48). Both types of receptors are present in the amygdala (57, 60). If the memory influence of peripheral E is indeed mediated by amygdala NE, it would be important to investigate the type of receptors involved. Previous studies have shown that amygdaloid β noradrenergic receptors are involved in the memory modulatory effects of intra-amygdala infused NE or peripherally injected E in normal animals (12, 15, 30), yet it is unknown whether the same also holds for the ADMX rats. It has been proposed that blocking presynaptic α_2 receptors in the amygdala would enhance NE release and thus improve memory (15). However, the role of amygdala ⁻stsynaptic α_1 noradrenergic receptors in the memory ˡulatory effects has not been formally elucidated. ⁻, the β receptor is known to couple with the Gs ›d its activation elevates intracellular cAMP ⁻ent evidence suggests that inhibitory ›ing involves activation of hippocampal ⁴enyl cyclase (7). In view of these cAMP into amygdala neurons ›ry or attenuate the memory deficit caused by depleting peripheral E. These possibilities were addressed by the present study.

The blood-brain-barrier may become defective under high blood pressure as a result of peripheral E administration (25). The aforementioned data, although consistent with that central NE mediates the effect of peripheral E on memory, are unable to rule out the hypothesis that some amount of E might leak into the brain and directly activate amygdala β receptors. To evaluate this possibility, we depleted amygdala NE by a neurotoxin —N-2-chloroethyl-N-ethyl-2-bromobenzylamine (DSP-4) (14, 26). If peripheral E requires amygdala NE to mediate its influences on memory, DSP-4 should abolish the effect of E on memory. On the other hand, if E directly activates β receptors in the amygdala, its effect on memory should persist after depletion of NE in the amygdala by DSP-4.

Extensive evidence has shown that training on aversive tasks may alter NE levels in brain regions (8, 18, 34, 53). Multi-trial visual discrimination learning enhances NE activities in regions including the pyriform cortex and amygdala (13). Training on a delayed response task also results in greater changes of the NE metabolism in the frontal cortex and the amygdala than in any other brain regions (54). Previous evidence has shown that the inhibitory avoidance training and/or peripheral E injections did alter global NE levels in the forebrain (18). In view of the finding that NE released in the central amygdala nucleus is correlated with peripheral E levels in tree shrews (11), we examined whether our inhibitory avoidance procedure would alter NE levels in the amygdala and whether superimposed E injections would further modulate such changes.

Materials and Methods

Subjects

Male Sprague-Dawley rats, about 4 months old, weighing about 300 grams were used in this study. After arriving from the breeding center, they were individually housed in animal rooms. Food and water were available all the time. A 12:12 light:dark cycle was adopted with lights on at 7:00 a.m. throughout the study.

Surgery

One month after arriving, rats were implanted with guide cannulae bilaterally into the amygdala. They were anesthetized with i.p. injection of sodium pentobarbital (45 mg/kg). To prevent respiratory congestion, atropine sulfate (0.4 mg/kg) was given 10 min before the anesthetics. To implant cannulae into

the amygdala, the anesthetized rat was mounted on a DKI-900 stereotaxic instrument, the coordinates were AP. - 1.0 mm, ML. ± 4.5 mm and DV. - 6.5 mm with the incisor bar was set at + 5.0 mm. Cannulae were made of 23 gauge stainless steel tubing with 0.33 mm inner diameter and a 0.63 mm outer diameter at a length of 15 mm. Two jewelry screws were implanted over the right frontal and the left posterior cortices serving as anchors. The whole assembly was affixed on the skull with dental cement. Intra-muscular injections of antibiotics (bicillin, 40,000 I.U.) were given at the end of surgery. Rats were kept warm until resurrection from the surgery. They recuperated for two weeks before any behavioral experiments.

Adrenal demedullation was performed in Experiment V to deplete adrenal E in groups of rats. Bilateral incisions were made on the flank of an anesthetized rat to expose the adrenal gland. The medulla tissue was removed by iris scissors and the remaining cortex was returned into the abdominal cavity. The wound was sutured. This procedure would deplete more than 95% of peripheral E (29). Sham operation followed the same procedure except that the adrenal medulla tissue was not removed.

Behavioral Task

The inhibitory avoidance apparatus was a trough-shape alley divided by a sliding door into a well-lit safe compartment and a dark shock compartment. The rat was placed into the lit side facing away from the door. As the rat turned around, the door was opened. After the rat stepped into the dark compartment, it received an inescapable footshock through a constant current shocker connected to a timer (Lafayette Instruments, Model 80240 and Model 58010). The shock intensity, which would be specified in each individual experiment, was calculated as the root mean square of the sinusoidal alternating currents. After shock administration, the rat was removed from the alley and returned to its cage. In the retention test given 24 hrs later, the rat was reintroduced into the alley and its latency to step into the shock compartment was taken as a measure of retention. If the rat did not step through in 10 min, the test trial was terminated and a ceiling score of 600 was assigned.

Drugs and Drug Administration

Norepinephrine hydrochloride (NE), isoproterenol, dl-propranolol, and 8-bromo-cAMP were obtained from Sigma (St. Louis, MO). Prazosin and DSP-4 were generous gifts from Dr. G. Novack of Allergan and Dr. F. Hock of Hoechst, respectively. They were dissolved into a specific brain buffer which in 100 ml contained 0.9 g of NaCl, 4.5 ml of 0.2 M Na_2HPO_4, and 0.95 ml of $NaH_2PO_4 \cdot 2H_2O$, which also served as the vehicle (Veh) for control infusion. If two drugs were infused into the amygdala of the same rat, they were dissolved into one solution and a total volume of 1 µl was given.

The intra-amygdala infusion device was constructed as follows: A piece of 0.5 m polyethylene tubing (PE-20, Clay Adams) was connected to a 10 µl Hamilton microsyringe on one end and cemented to a 30 gauge dental needle on the other. The syringe and the tubing were first filled with distilled water. Drug solutions were then introduced from the injection needle and separated by a tiny air bubble from the distilled water. Drug infusion was administered to a conscious rat shortly before or after the behavioral test. Care was taken to minimize stressing the animal. The rat was gently held and the injection needles were inserted into the cannulae with the stylet removed. To facilitate diffusion of drugs, the infusion needle protruded 1.5 mm beyond the tip of the cannulae. The rat was then placed into a small cardboard container for restraining from drastic movement. Intra-cerebral infusion was administered bilaterally through a syringe pump (Sage Instrument, Model 355) at a rate of 1.0 µl/1 min. The infusion volume on each side was 1.0 µl for the amygdala. After infusion, the needle remained in the cannula for one additional min before withdrawn and the stylet was replaced immediately to prevent back flow.

Histology Verification

At the conclusion of each experiment, animals were sacrificed with an overdose of sodium pentobarbital (50 mg per rat, i.p.) and perfused through the heart with physiological saline followed by 10 % formalin. The brain was then removed, stored in formalin for at least 48 hrs. The brains were sectioned (40 µm). The brain slices stained with cresyl violet. Placements of the cannulae were recorded by projecting the stained slides onto a brain atlas chart (41).

HPLC-EC Assays of Monoamines

Brain tissues from a group of rats receiving footshock, E injections or intra-amygdala infusion of DSP-4 were subjected to monoamine assays. Rats were decapitated and their brains were quickly removed on ice. The following brain regions were dissected out by following a previously described procedure (16): The frontal cortex, amygdala, hypothalamus, hippocampus, midbrain, and pons-medulla. The dissected brain regions were immediately frozen on dry ice, weighed, and stored at -75 °C until monoamine assays. The procedure for

simultaneous determination of monoamines was modified from that described previously (35). Briefly, the tissue was homogenized in HCl with 100 ng of dihyroxybenzylamine (DHBA) serving as the internal standard and monoamines were extracted by a butanol method. Twenty microliters of the aqueous extract were injected into a reverse phase column (Ultrasphere-ODS, 4.6×25 cm, 5 µ, Beckman) through a Reodyne injector. The mobile phase was a 0.04 M sodium citrate/citric acid buffer (pH 5.0) containing 0.8% tetrahydrofuran. The flow rate was 1 ml/min. Monoamines were detected by an electrochemical detector (BAS, LC-4B, West Lafayette) with a glassy-carbon electrode. The applied voltage was set at 0.6 V versus the Ag/AgCl reference electrode. The dopamine (DA), serotonin (5-HT) and NE levels in the sample were calculated by calibration with external standards according to the relative peak heights (in comparison with the internal standard DHBA). The concentration was expressed as nanograms per gram of wet tissue.

Data Analysis

Because the data distribution from the inhibitory avoidance task was truncated at 600, medians and interquartile ranges were used to express the central and dispersion tendencies, respectively, of all groups. The data were subjected to nonparametric tests. The Kruskal-Wallis one-way ANOVA was used to evaluate the overall significant difference among various groups. The Mann-Whitney U-test was used to assess the difference between two groups in paired comparisons. Differences in the monoamine levels were evaluated by the student t-tests.

Results

Experiment I: Posttraining Blockade of Amygdala β Receptors Impaired Retention.

Ten groups of rats were trained with a 1.7 mA/1 s footshock which was routinely adopted for demonstration of an impairing effect. They received immediately posttraining intra-amygdala infusion of Veh or 0.2, 1.0 or 5.0 µg of the α_1 blocker prazosin or the β blocker propranolol. Fig. 1 shows that both drugs appeared to cause a dose-dependent memory deficit. However, a Kruskal-Wallis one-way ANOVA indicated that differences among various prazosin-treated groups failed to reach statistical significance ($H(3) = 6.2$, $p > 0.1$), although the difference between the 5.0 µg prazosin group and the Veh group approached statistical significance ($U = 13.5$, $0.05 < p < 0.10$). In contrast, there

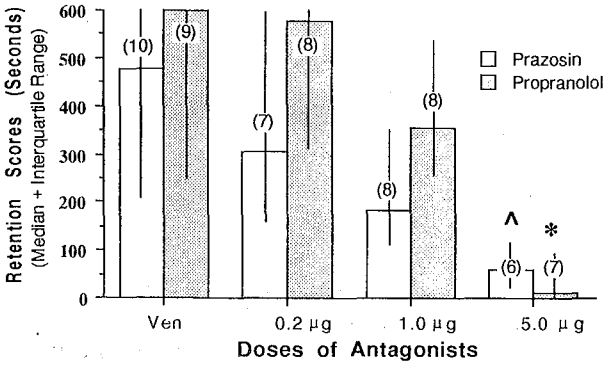

Fig. 1. Effects of immediate posttraining intra-amygdala infusion of the α_1- or β-noradrenergic antagonist (prazosin or propranolol, respectively) on retention of the inhibitory avoidance response. * $p < 0.05$, ^ $0.05 < p < 0.10$; different from the corresponding Veh group.

were significant differences among the propranolol-treated groups ($H(3) = 10$, $p < 0.02$). Further paired comparisons indicated that the group receiving 5.0 µg propranolol had significantly lower retention scores than the Veh group ($U = 7.5$, $p < 0.05$).

Experiment II: Posttraining Stimulation of Amygdala β Receptors Enhanced Retention.

Ten groups of rats were trained with a 1.0 mA/1 s footshock, which was routinely used for demonstration of memory facilitation. Immediately after training, they received intra-amygdala infusion of Veh or 0.05, 0.2 or 1.0 µg of the α_1 agonist phenylephrine or the β agonist isoproterenol. Fig. 2 shows that isoproterenol, but not phenylephrine, enhanced the otherwise feeble memory. A Kruskal-Wallis one-way ANOVA revealed no significant

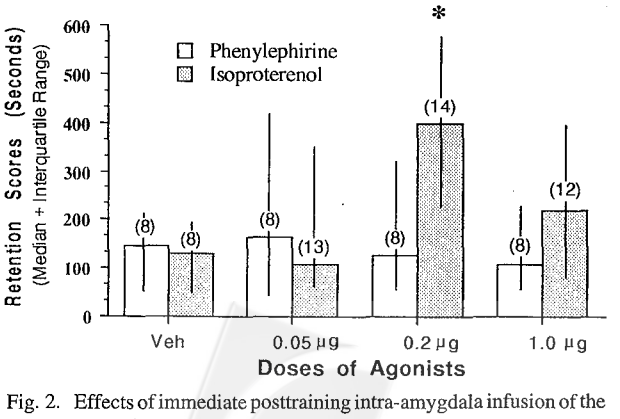

Fig. 2. Effects of immediate posttraining intra-amygdala infusion of the α_1- or β-noradrenergic agonist (phenylephrine or isoproterenol, respectively) on retention of the inhibitory avoidance response. * $p < 0.05$ different from the corresponding Veh group.

difference among the various phenylephrine-treated groups ($H(3) = 0.2$, $p > 0.1$). On the other hand, the Kruskal-Wallis one-way ANOVA revealed a significant difference among the various isoproterenol-treated groups ($H(3) = 8.3$, $p < 0.05$). Further paired comparisons indicated that rats given 0.2 µg of isoproterenol had significantly better retention than the Veh group ($U = 21.5$, $p < 0.02$), whereas rats receiving the higher or lower dose of isoproterenol had retention scores not significantly different from those of the Veh group ($U = 46.5$ & 36, $p > 0.2$, for the 0.05 & 1.0 µg group, respectively).

Experiment III: Posttraining Intra-Amygdala Infusion of 8-Bromo-cAMP Enhanced Retention.

Five groups of rats were trained as in Experiment II. Immediately after training, four groups of rats received intra-amygdala infusion of Veh, 0.2, 1.0 or 5.0 µg 8-bromo-cAMP, an analog of cAMP which readily entered the cell when given extracellularly. An extra group received 5.0 µg 8-bromo-cAMP 6 hrs after training. As indicated in Fig. 3, 8-bromo-cAMP infused into the amygdala after training caused a dose- and time-dependent memory facilitation. A Kruskal-Wallis one-way ANOVA revealed significant overall differences among various groups ($H(4) = 9.7$, $p < 0.05$). Further paired comparisons indicated that only the group receiving 5.0 µg cAMP immediately after training had better retention than the Veh group ($U = 18$, $p < 0.01$). However, the group receiving 8-bromo-cAMP 6 hrs after training did not differ from the Veh group and performed significantly poorer than the group receiving the same dose of 8-bromo-cAMP immediately after training ($U = 23$, $p < 0.05$).

Experiment IV: Effects of Intra-Amygdala Infused NE on Memory Involved Amygdala β Receptors, but not α_1 Receptors.

Four groups of rats were trained as in the previous experiment. Immediately after training, they received one of the following treatments: Veh, 0.2 µg NE, 0.2 µg NE plus 0.2 µg prazosin, or 0.2 µg NE plus 0.2 µg propranolol (denoted as the Veh, NE, NE/Praz and NE/Prop groups, respectively). Fig. 4 shows that, in replicating previous findings, posttraining intra-amygdala infusion of NE produced a marked memory enhancing effect and this effect was readily attenuated by simultaneous infusion of propranolol, but not by infusion of prazosin. A Kruskal-Wallis one-way ANOVA indicated a significant overall difference among various groups ($H(3) = 15.6$, $p < 0.005$). Further comparisons indicated that the NE group had significantly better retention scores than the Veh group ($U = 9$, $p < 0.001$). The NE/Praz group also had significantly better retention scores than the Veh group ($U = 12$, $p < 0.05$) and did not differ from the NE group ($U = 41$, $p > 0.5$). On the other hand, the NE/Prop group had retention scores not significantly different from the Veh group ($U = 43.5$, $p < 0.2$), but significantly lower than the NE group ($U = 29$, $p < 0.01$) and the NE/Praz group ($U = 18$, $p < 0.05$).

Experiment V: The Memory Normalizing Effect of Peripheral E in the ADMX Rats Involved Amygdala β Receptors, but not α_1 Receptors.

Rats received 1.7 mA/1 s footshock training such that the deleterious effect of adrenal demedullation could be easily demonstrated. Immediately after training, a sham operated group

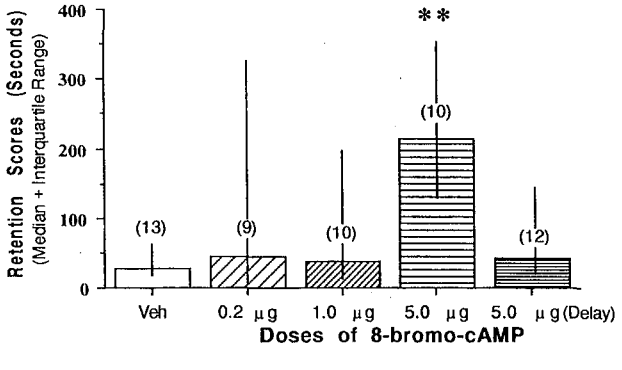

Fig. 3. The effect of posttraining intra-amygdala infusion of 8-bromo-cAMP on retention of the inhibitory avoidance response. The delayed infusion was given 6 hrs after training. ** $p < 0.01$ different from the Veh group.

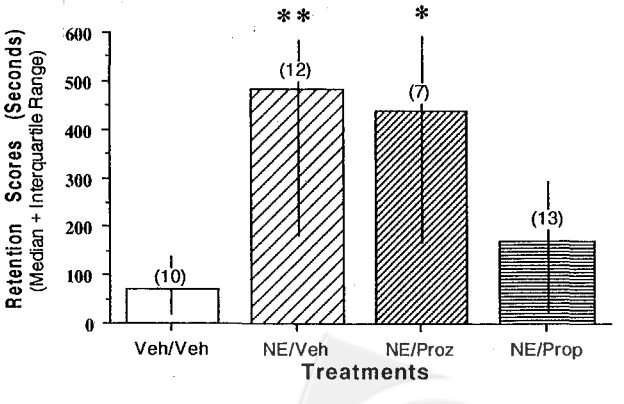

Fig. 4. The memory enhancing effect of norepinephrine was attenuated by the β blocker propranolol, but not by the α_1 blocker prazosin. ** $p < 0.01$; * $p < 0.05$ different from the Veh/Veh group and the NE/Prop group.

received intra-amygdala infusion of Veh and then a subcutaneous (s.c.) injection of saline (Sal). Five ADMX groups received one of the following combined treatments (amygdala/peripheral): Veh/Sal, Veh/E, prazosin/E, propranolol/E or 8-bromo-cAMP/Sal. The doses of prazosin and propranolol were 0.2 μg and that of 8-bromo-cAMP was 5.0 μg. The dose of E was 0.1 mg/kg. Fig. 5 shows that depletion of peripheral E by adrenal demedullation caused a severe retention deficit, and that was attenuated by peripheral E injection or by intra-amygdala infusion of 8-bromo-cAMP given immediately after training. The memory normalizing effect of E was blocked by the β blocker, but not by the α_1 blocker, infused into the amygdala.

A Kruskal-Wallis one-way ANOVA revealed a significant difference among various groups ($H(5) = 26.26$, $p < 0.0001$). Further paired comparisons indicated that for the Veh/Sal treated groups, retention scores of the ADMX rats were significantly lower than those of the Sham rats ($U = 41$, $p < 0.01$). For the ADMX rats, the Veh/E group or the 8-bromo-cAMP/Sal group did significantly better than the Veh/Sal group ($U = 41$ or 51, respectively, $p < 0.005$) and did not differ from the Sham controls. The effect of E was reversed by 0.2 μg propranolol, but not by 0.2 μg prazosin, infused into the amygdala: In the ADMX rats, the Prop/E group did not differ from the Veh/Sal group ($U = 40$, $p > 0.1$), but performed significantly poorer than the Veh/E group ($U = 6$, $p < 0.001$) and the Sham controls ($U = 6$, $p < 0.005$). Contrarily, the Praz/E group performed significantly better than the Veh/Sal group ($U = 32$, $p < 0.01$) and the Prop/E group ($U = 6$, $p < 0.005$), but did not differ from the Veh/E group and the Sham controls.

Experiment VI: The Memory Modulatory Effect of Peripheral E was Blocked by Depleting Amygdala NE.

Two weeks before being trained with an 1.0 mA/1 s footshock, eight groups of rats received intra-amygdala infusion of Veh or 3.0 μg DSP-4 which selectively depletes NE. Immediately after training, they were injected (s.c.) with either saline or E at doses of 0.01, 0.1 and 0.5 mg/kg. Fig. 6 shows that in rats with normal amygdala NE functioning, posttraining s.c. injection of E caused dose-dependent biphasic effects on retention: 0.1 mg/kg enhanced memory, but 0.5 mg/kg impaired memory. Both effects were completely abolished after depletion of NE in the amygdala by DSP-4.

A Kruskal-Wallis one-way ANOVA showed significant differences among the Veh-pretreated groups ($H(3) = 30.9$, $p < 0.0001$). Further paired comparisons indicated that the 0.1 mg/kg E group had significantly better retention than the corresponding Veh controls ($U = 2$, $p < 0.001$), whereas the 0.5 mg/kg E group had significantly poorer retention than the corresponding Veh group ($U = 23$, $p < 0.005$). Intra-amygdala infusion of DSP-4 by itself had no significant effect in the saline injected rats. No significant difference was found among the various DSP-4-pretreated groups ($H(3) = 1.9$, $p > 0.5$). Retention scores of the DSP-4 pretreated rats given 0.1 mg/kg or 0.5 mg/kg E did not differ from those of the DSP-4/saline controls, but were significantly lower or higher, respectively, than those of the corresponding Veh-pretreated group receiving the same dose of E ($U = 12$ or 21, $p < 0.01$; respectively). The NE contents (mean ± standard error) of the amygdala in the Veh and DSP-4 treated animals were 537 ± 19 ng/g tissue and 453

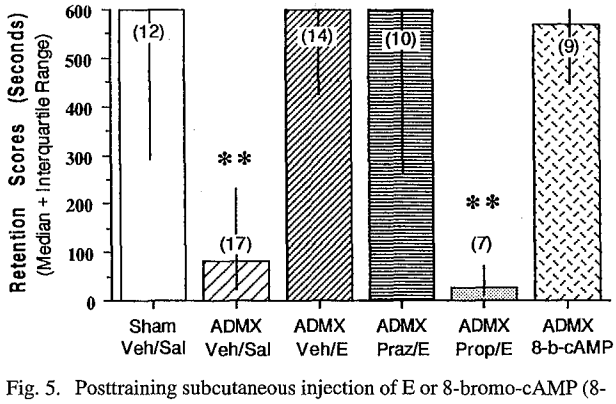

Fig. 5. Posttraining subcutaneous injection of E or 8-bromo-cAMP (8-b-cAMP) ameliorated the memory deficit in the adrenal demedullated (ADMX) rats. The effect of E was abolished by posttraining intra-amygdala infusion of the β blocker propranolol but not by the α_1 blocker prazosin. ** $p < 0.01$ different from all other groups.

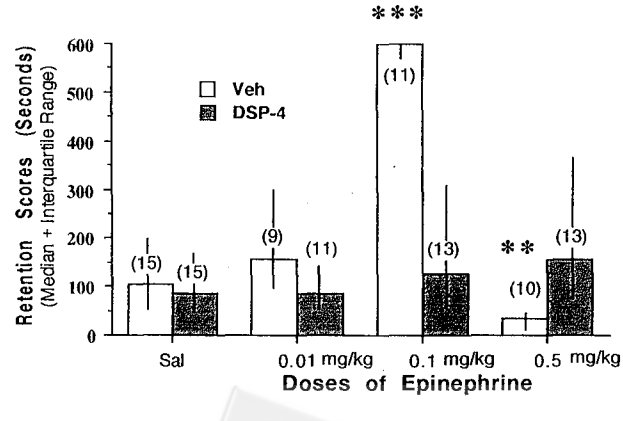

Fig. 6. Pretreating the amygdala with DSP-4 attenuated both the memory enhancing effect of epinephrine at a low dose (0.1 mg/kg) and the memory impairing effect of epinephrine at a high dose (0.5 mg/kg). *** $p < 0.001$, ** $p < 0.01$ different from the Veh/Sal group and the corresponding DSP-4-treated group.

± 16 ng/g tissue, respectively. The difference was significant ($t = 3.37$, $p < 0.001$).

Experiment VII. Footshock or Peripheral E Injection Failed to Alter NE Levels in Various Brain Regions.

Four groups of rats received one of the following treatments: no footshock plus saline (NS/Sal), footshock plus saline (FS/Sal), no footshock plus E (NS/E), footshock plus E (FS/E). The shock was 1 mA/1 s given in the dark compartment. Rats receiving no footshock were also placed in the box for an equal amount of time. They then received an s.c. injection of saline or 0.1 mg/kg E and were decapitated 10 min later.

The NE, DA and 5-HT levels in various brain regions are shown in Table 1, 2 and 3, respectively. The tissue NE, DA and 5-HT concentrations were not altered by any of the treatments. While the adopted method is capable of detecting E, no significant amount of E was found in various brain regions after different treatments.

Histological Verifications

The distribution of cannula tips is shown in Fig. 7. As shown in the figure, the tips of cannulae were located at the various nuclei of the amygdala, but mostly around the central and basolateral amygdala nuclei. No significant correlation was found between the retention scores of animals and the locations of cannulae within the amygdala.

Table 1. Norepinephrine levels in various brain regions after footshock and/or peripheral epinephrine injection

	no shock/sal	no shock/epi	footshock/sal	footshock/epi
amygdala	534 ± 53@	563 ± 46	517 ± 47	518 + 128
hypothalamus	2114 ± 381	2097 ± 235	2255 ± 408	2174 ± 308
frontal cortex	194 ± 16	183 ± 19	208 ± 14	181 ± 25
hippocampus	519 ± 144	544 ± 122	596 ± 144	639 ± 80
pons-medulla	755 ± 56	771 ± 159	795 ± 206	741 ± 121
midbrain	517 ± 32	443 ± 95	536 ± 120	530 ± 121

@ X + S.E. (ng/g wet tissue)

Table 2. Dopamine levels in various brain regions after footshock and/or peripheral epinephrine injection

	no shock/sal	no shock/epi	footshock/sal	footshock/epi
amygdala	581 ± 201	684 ± 237	506 ± 176	595 + 91
hypothalamus	490 ± 74@	416 ± 80	405 ± 98	499 ± 110
frontal cortex	538 ± 106	525 ± 123	536 ± 60	525 ± 151
hippocampus	39 ± 39	49 ± 35	42 ± 42	34 ± 16
pons-medulla	59 ± 29	30 ± 13	42 ± 15	44 ± 5
midbrain	517 ± 32	171 ± 54	186 ± 69	176 ± 26

@ X + S.E. (ng/g wet tissue)

Table 3. Serotonin levels in various brain regions after footshock and/or peripheral epinephrine injection

	no shock/sal	no shock/epi	footshock/sal	footshock/epi
amygdala	659 ± 223	506 ± 217	548 ± 101	595 + 91
hypothalamus	1042 ± 238	1179 ± 167	818 ± 272	790 ± 154
frontal cortex	114 ± 41	138 ± 87	246 ± 174	206 ± 42
hippocampus	245 ± 143	300 ± 111	178 ± 136	325 ± 212
pons-medulla	132 ± 70	121 ± 67	103 ± 49	257 ± 129
midbrain	272 ± 162	368 ± 160	327 ± 210	317 ± 110

@ X + S.E. (ng/g wet tissue)

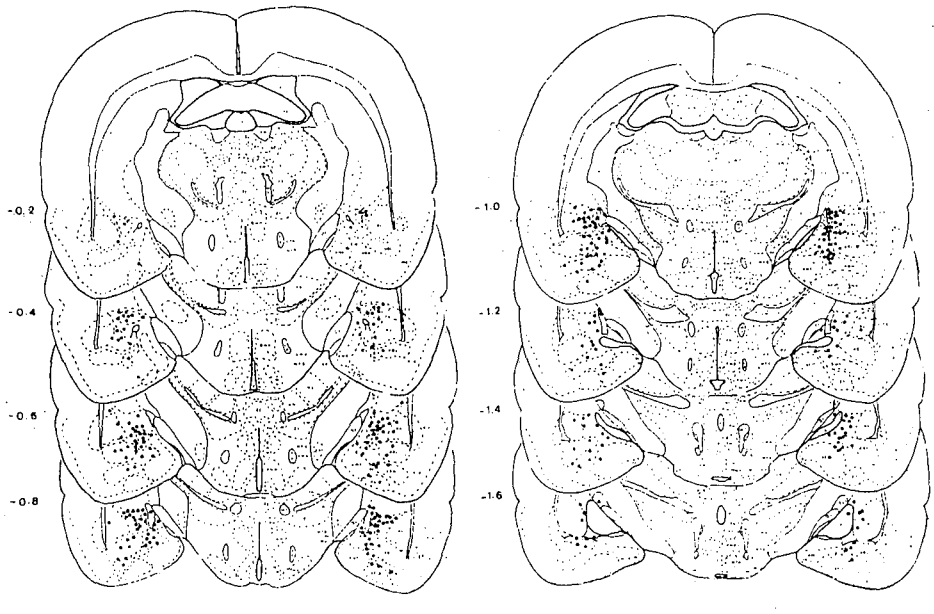

Fig. 7. The distribution of cannula tips in the experimental groups of animals.

Discussion

The major findings of the present study can be recapitulated as follows: First, in the inhibitory avoidance task, immediate posttraining intra-amygdala infusion of the β-noradrenergic agonist (isoproterenol) or antagonist (propranolol) produced, respectively, memory enhancement or impairment in a dose-dependent manner. The memory enhancing effect of NE was blocked by propranolol and mimicked by isoproterenol and 8-bromo-cAMP. Second, depleting peripheral E by adrenal demedullation created a retention deficit. This deficit could be attenuated by supplementing E to the periphery or 8-bromo-cAMP to the amygdala. Pretreating the amygdala with the NE neurotoxin DSP-4 abolished memory modulating effects of peripheral E. In contrast, amygdala α_1-noradrenergic functioning appeared not to be involved in these effects. Finally, neither footshock nor peripheral E injection altered discernibly the static state monoamine levels, as assessed 10 min after the treatment in various brain regions including the amygdala.

In the present study, drug treatments were administered shortly after training, therefore, the effects on retention cannot be attributed to influences on sensorimotor ability during acquisition. While intra-amygdala administration of DSP-4 was a pretraining treatment, our recent findings (Liang, in preparation) indicated that it had no effect on either shock sensitivity or locomotor activity of rats. The memory enhancement produced by immediate posttraining infusion of 8-bromo-cAMP is not likely due to its influence on motor performance extended to the retention test, otherwise a delayed treatment should be more effective than an immediate treatment.

The histology indicated that cannula tips were distributed within the amygdala and mostly around basal amygdala nuclei, where NE terminals from the brainstem innervate (40). While it has been reported that the central amygdaloid nucleus was the most effective site for NE influences on memory processes (15), no differential effects were found for various infusion sites in the present study. Previous evidence has ruled out that the effect could be due to diffusion of noradrenergic drugs into the striatum (30). Evidence has shown that NE in the dorsal hippocampus is involved in memory formation for the inhibitory avoidance task (28). We infused NE into the ventral hippocampus over a wide dose range and under various training conditions but failed to find any significant memory enhancing effect (data not shown). Therefore, the effect of intra-amygdala infused noradrenergic drugs is not likely due to drug diffusion into the adjacent ventral hippocampus.

This study replicated that posttraining intra-amygdala infusion of propranolol, at a high dose (5.0 μg), impaired retention. The present results extended previous ones by showing that propranolol at a much lower dose (0.2 μg), which by itself did not affect retention, readily attenuated the memory enhancement induced by NE. In addition, immediate posttraining

intra-amygdala infusion of isoproterenol or 8-bromo-cAMP produced a memory facilitation similar to that produced by NE. These findings, taken together, suggest that, endogenous NE released in the amygdala during training activates the β receptor-Gs protein-adenyl cyclase signal transduction cascade which plays an important role in memory modulation, as what has also been shown in the hippocampus (7).

The present results indicated prazosin (0.2 μg) failed to attenuate the memory enhancing effect of NE. This finding could be viewed as evidence against a role of amygdala α_1-noradrenergic receptors in the memory modulatory influences of NE. This suggestion is consistent with the finding that posttraining intra-amygdala infusion of phenylephrine produced no memory enhancing effect. It is paradoxical that prazosin by itself at a high dose (5.0 μg) appeared to impair retention. This might be due to some nonspecific effects of prazosin at high doses and should be clarified in the future. In view of the recent report that clonidine infused into the frontal cortex of monkeys affected performance in a delayed alternating task (3), the role of amygdala α_2 noradrenergic receptors in memory processing should be pursued in the future.

Consistent with previous findings (4, 31), the present results showed that depleting peripheral E by adrenal demedullation produced a profound retention deficit. Peripheral injection of E has been shown to facilitate memory in otherwise untreated animals and attenuate the memory deficit in ADMX rats (29), which were fully replicated in this study. More significantly, the present results showed that the effect, on retention, of peripheral E in ADMX rats was blocked by intra-amygdala injections of propranolol rather than prazosin. These findings suggest that the action of amygdala NE on β, but not α_1, receptors, is critically involved both the memory facilitating effect and memory normalizing effect of peripheral E. This suggestion is further supported by the finding that intra-amygdala infusion of 8-bromo-cAMP also ameliorated the retention deficit of ADMX rats.

A previous study has shown that peripheral administration of DSP-4 impaired memory (2). That intra-amygdala infusion of DSP-4 failed to impair memory in the present study might have been viewed as evidence against a critical role of amygdala NE in memory formation. However, because animals were trained under moderate footshock which generated low retention, a floor effect might conceal the detrimental influence of DSP-4 . In a separate study, we have demonstrated a memory impairing effect of DSP-4 at a higher dose (30 mg) on rats trained under a higher footshock (1.7 mA/1 s) (Liang, in preparation), which is consistent with the endogenous memory modulatory role of NE.

A single dose of DSP-4 attenuated both enhancing and impairing effects of low and high doses of E. Thus, the attenuation could not be easily attributed to a general facilitating or debilitating influence of DSP-4. This finding rules out the possibility that E might have leaked into the amygdala and directly stimulated postsynaptic β receptors to enhance memory. Blocking amygdala α_2 receptors enhanced memory presumably by enhancing NE release (15). The memory impairing effect of E at a high dose might be due to E acting directly on amygdala α_2 presynaptic receptors to block NE release. This possibility is not plausible in view of no trace amount of E detected in various brain regions of rats after peripheral E injection. Thus, the influence of E on amygdala NE functioning is most likely to be indirect.

In contrast with previous findings that various treatments induced profound changes of the global NE level in the forebrain (18), Exp. VII failed to find significant changes of the amygdala NE level after footshock or peripheral injections of E. Previous studies have shown that footshock training or 4-OH-amphetamine, a peripherally acting memory enhancer, alters DA and 5-HT levels in certain brain regions (1, 35), the present results show that neither E nor footshock alter DA or 5-HT levels. The lack of effect of E injections on amygdala NE levels might be viewed as evidence for that the amygdala NE system does not normally mediate influences of peripheral E on memory. Under this interpretation, results from those manipulative studies could merely be interpreted as the consequences of pharmacological treatments and have no bearings upon the natural physiological mechanism. However, given the rapid turn-over of NE, footshock or E could induce amygdala NE functional changes which is not well reflected in the postmortem static-state NE level. This conjecture is viable in view of the results that 3.0 μg of DSP-4 reduced only less than 20% of the static state amygdala NE level but was sufficient to abolish the memory modulatory effect of E. The single time point (10 min after treatments) sampled by the present study may also contribute to the failure of detecting any changes. In view of these issues, to monitor the in vivo monoamine release in the amygdala or other brain structures for a period of time after the footshock or E injection is needed for a clear resolution.

If peripheral E indeed affects memory through the amygdala NE system, it would be interesting to contemplate the mechanism of how this is accomplished. E in the periphery could affect brain NE functions through its influences on the regional cerebral metabolism such as altering the glucose supply (19) or by acting on certain blood-brain-barrier leakage areas, such as the area postrema.

Alternatively, peripheral E may affect amygdala NE activities through neural inputs (50). Noradrenergic projections from the brainstem including the locus coeruleus and the nucleus of the solitary tract innervate the amygdala (40, 43). Anatomical and physiological evidence indicates that brain stem noradrenergic nuclei receive visceral inputs (49, 51). Therefore, E, given systemically, may activate visceral afferents which in turn excite NE nuclei in the pons or medulla (22, 59) and result in release of NE in the amygdala. In accordance with such a view, transecting the VAF which carries NE fibers into the amygdala readily attenuated the memory modulatory effect of peripheral injections of E (33). The role of brainstem noradrenergic nuclei in mediating the effect is presently being investigated in this laboratory.

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